

WEST**Freeform Search****Database:**

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USPT,PGPB,JPAB,EPAB,DWPI	14 same recombinant same human	5	<u>L11</u>
USPT,PGPB,JPAB,EPAB,DWPI	14 same "fusion"	5	<u>L10</u>
USPT,PGPB,JPAB,EPAB,DWPI	14 same "epo"	5	<u>L9</u>
USPT,PGPB,JPAB,EPAB,DWPI	14 same "sf"	4	<u>L8</u>
USPT,PGPB,JPAB,EPAB,DWPI	14 same "gm-csf"	9	<u>L7</u>
USPT,PGPB,JPAB,EPAB,DWPI	14 same "g-csf"	6	<u>L6</u>
USPT,PGPB,JPAB,EPAB,DWPI	14 same (medium or media)	9	<u>L5</u>
USPT,PGPB,JPAB,EPAB,DWPI	"flt3-ls"	16	<u>L4</u>
USPT,PGPB,JPAB,EPAB,DWPI	11 or 12	71	<u>L3</u>
USPT,PGPB,JPAB,EPAB,DWPI	beckman-m\$.in.	26	<u>L2</u>
USPT,PGPB,JPAB,EPAB,DWPI	lyman-s\$.in.	45	<u>L1</u>

=> d his

(FILE 'HOME' ENTERED AT 10:29:27 ON 01 MAY 2001)

FILE 'MEDLINE' ENTERED AT 10:29:31 ON 01 MAY 2001

L1 417 S FLT3-L OR FLT3-LIGAND/AB,BI
L2 74 S L1 AND G-CSF/AB,BI
L3 18 S FLT3/FLK2 LIGAND/AB,BI

FILE 'STNGUIDE' ENTERED AT 10:32:42 ON 01 MAY 2001

FILE 'MEDLINE' ENTERED AT 10:34:44 ON 01 MAY 2001

L4 423 S L1 OR L3
L5 74 S L2 AND L4
L6 99 S L4 AND GM-CSF/AB,BI
L7 17 S L4 AND SF/AB,BI
L8 30 S L4 AND EPO/AB,BI
L9 24 S L4 AND FUSION/AB,BI

FILE 'MEDLINE, EMBASE, BIOSIS, INPADOC, CAPLUS' ENTERED AT 10:39:12 ON

01

MAY 2001

E LYMAN S/AU
L10 409 S E3 OR E6
L11 170 S L10 AND L4
L12 1799 S L4
E LYMAN STEWART/AU
L13 236 S E1-E5
L14 98 S L13 AND L4
L15 60 DUP REM L14 (38 DUPLICATES REMOVED)
E BECKMAN M/AU
L16 73 S E3 OR E12
E BECKMAN M PATRICIA/AU
L17 5 S E3
L18 78 S L16 OR L17
L19 1 S L18 AND L4
L20 383 S L6
L21 177 DUP REM L20 (206 DUPLICATES REMOVED)
L22 59 S L7
L23 23 DUP REM L22 (36 DUPLICATES REMOVED)
L24 104 S L8
L25 38 DUP REM L24 (66 DUPLICATES REMOVED)
L26 82 S L9
L27 49 DUP REM L26 (33 DUPLICATES REMOVED)

WEST

Generate Collection

L11: Entry 1 of 5

File: USPT

Feb 20, 2001

DOCUMENT-IDENTIFIER: US 6190655 B1

TITLE: Methods of using Flt-3 ligand for exogenous gene transfer

BSPR:

Transformed yeast host cells are preferably employed to express flt3-L as a secreted polypeptide in order to simplify purification. Secreted recombinant polypeptide from a yeast host cell fermentation can be purified by methods analogous to those disclosed by Urdal et al. (J. Chromatog. 296:171, 1984). Urdal et al. describe two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

DEPR:

Prior to cell collection, it may be desirable to mobilize or increase the numbers of circulating PBPC and PSC. Mobilization can improve PBPC and PSC collection, and is achievable through the intravenous administration of flt3-L to the patients prior to collection of such cells. Other growth factors such as CSF-1, GM-CSF, SF, G-CSF, EPO, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, GM-CSF/IL-3 fusion proteins, LIF, FGF and combinations thereof, can be likewise administered in sequence, or in concurrent combination with flt3-L. Mobilized or non-mobilized PBPC and PSC are collected using apheresis procedures known in the art. See, for example, Bishop et al., Blood, vol. 83, No. 2, pp. 610-616 (1994). Briefly, PBPC and PSC are collected using conventional devices, for example, a Haemonetics Model V50 apheresis device (Haemonetics, Braintree, Mass.). Four-hour collections are performed typically no more than five times weekly until approximately 6.5.times.10.sup.8 mononuclear cells (MNC)/kg patient are collected. Aliquots of collected PBPC and PSC are assayed for granulocyte-macrophage colony-forming unit (CFU-GM) content by diluting approximately 1:6 with Hank's balanced salt solution without calcium or magnesium (HBSS) and layering over lymphocyte separation medium (Organon Teknika, Durham, N.C.). Following centrifugation, MNC at the interface are collected, washed and resuspended in HBSS. One milliliter aliquots containing approximately 300,000 MNC, modified McCoy's 5A medium, 0.3% agar, 200 U/mL recombinant human GM-CSF, 200 u/mL recombinant human IL-3, and 200 u/mL recombinant human G-CSF are cultured at 37 C in 5% CO.sub.2 in fully humidified air for 14 days. Optionally, flt3-L or GM-CSF/IL-3 fusion molecules (PIXY 321) may be added to the cultures. These cultures are stained with Wright's stain, and CFU-GM colonies are scored using a dissecting microscope (Ward et al., Exp.

are scored using a dissecting microscope (Ward et al., Exp. Hematol., 16:358 (1988). Alternatively, CFU-GM colonies can be assayed using the CD34/CD33 flow cytometry method of Siena et al., Blood, Vol. 77, No. 2, pp 400-409 (1991), or any other method known in the art.